Supplemental material (FOR Publication)

Supplemental Tables

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Table S1. Oligomers used in the LbRC assay

	Name	Sequence	Hits/Targets	Target comments
Forward primers	BU4L	CtCCTACGGGaGGCaGCA	2391696	Broad
	BU4L3	CaCCTACGGGtGGCaGCA	20244	Verrucomicrobi
	BU4L2	C-CCTACGGGgGGCaGCA	1631	Aquificae
	BU4LC	CtCCTACGGGaGGCtGCA	391	Chlamydiaceae
			2413957	69%
Reverse primers	BU6Rr	GACTACcrGGG-TATcTAAkCCtG	2058853	Broad
	BU6R4	GACTACcgGGG-TATcTAAtCC	124439	Broad
	BU6RU	GGACTACTAGGGTATcTAAtCCt	31719	Tenericutes, Proteobacteria, Bacteroides
	BU6R2	GACTACagGGG-TATcTAAtCC	22633	Cyanobacteria
	BU6RTB	GACTACccGGG-TATyTAAtCCgG	15971	miscellaneous
	BU6R3	GACTACcaGGGgTATcTAAtCC	4163	Proteobacteria, Firmicutes, Actinobacteria
			2257778	65%
Lactobacillus blocking oligomers	LBB3p	GGCAGCAGTAGGGAATCTTCC*AT*p	40339	87%
	LBB4p	GTTCGCTACCCATGCTTTCGAGC*CTCT*p	21925	49%

Primer sequences were derived from alignments of target and non-target entries in the Ribosome Database website from Michigan State University. Lowercase denotes polymorphic bases among primer sets. Numbers of target hits of each sequence used default website settings. Red letters denote bases that overlap primer sequences; italics denote mismatches to target sequence and "p" the 3' phosphate group that together minimize extension from the blocking oligomers. Both LBB3p and LBB4p complement all vaginal *Lactobacillus* species. Percentages are based on ratios of perfect complements to total targets in the RDP database. Among vaginal species, LBB3p also complements 95% of *Staphylococcus* entries. The program, after an initial denaturation at 95°C for 60s, was 40 cycles: 30s at 94°C, 15s at 74°C 20s at 56°C and 30s at 72°C. Melt curves were determined from 80°C to 93°C, reading fluorescence of Syto 9 at 0.2°C intervals. PCR reactions were in 10 mM Tris pH 8.3, 50 mM KCl, 3 mM MgCl2, 200 µM each dNTP, 0.23

μM primer, 37.5 nM Syto 9, and were performed in Biorad CFX Connect thermocyclers. Cq values were reset to default limit of detection, 38, if the initial Cq was above 38 and/or if all Tm values were less than 83°C. Reactions were recorded as unscoreable if their unblocked Cq was 33 or higher, as this indicates poor sample yield and precludes a meaningful determination of ΔCq. Positive and negative controls were included on each run. Samples were checked for PCR inhibitors by checking that Cq values of an external spike into the samples had Cq values within a standard deviation of 5 replicates of the spike alone.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis Nucl. Acids Res. 42(Database issue):D633-D642; doi: 10.1093/nar/gkt1244.

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Table S2. Percent agreement of ΔCq and Tm values in duplicate runs

Plate	dCq	Tm Dominant	Tm Sub-Dominant
15	91	88	95
28	74	98	99
77	91	88	95
171	98	97	89
191	96	88	98

ΔCq values were scored as in agreement if both were ≤ 2.5 or both were > 2.5. Tm values were scored as in agreement if they differed by ≤ 0.8 °C. Each plate DNA from swabs makes approximately 48 dCq and 96 Tm comparisons.

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Supplemental Figures

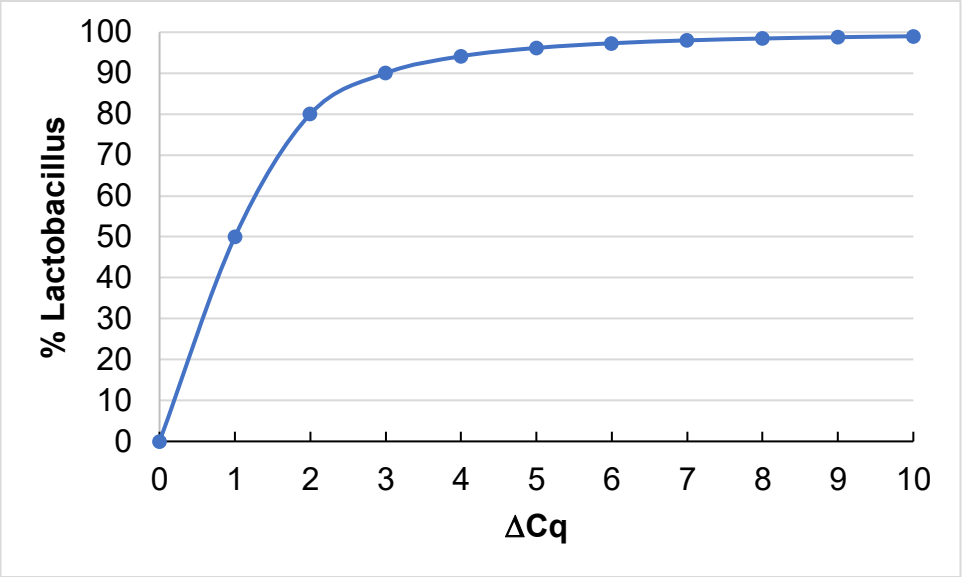

Fig. S1. Relationship between ΔCq and % *Lactobacillus*. Values are theoretical based on the equation 100*ΔCq^2)/(ΔCq^2+1). Values are most intuitive at ΔCq=1, since 50% *Lactobacillus* means that inhibiting amplification of half of the species in a sample should increase the Cq value by 1.

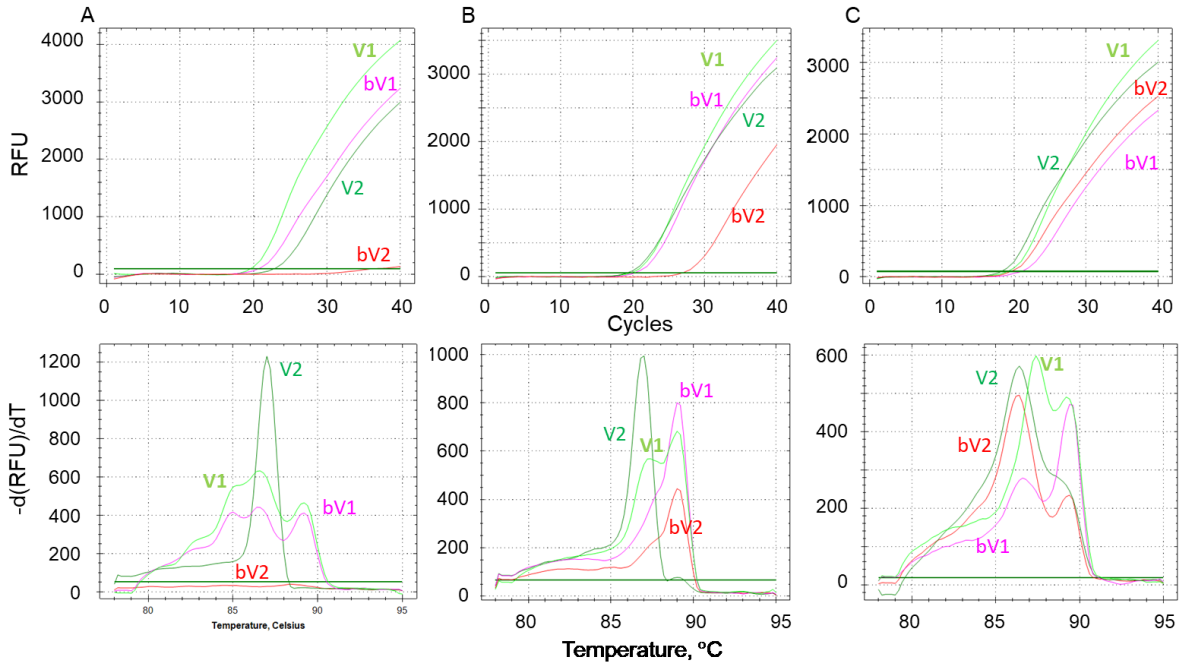

Fig. S2. LbRC analysis: qPCR of representative remission (A), recurrent (B), and refractory (C) responses to treatment. Upper panels are amplification curves, lower panels are derivative melt curves. Acute BV patients at visit 1 (V1), amplified without or with (bV1) *Lactobacillus* blockers.

Fig. S2 shows amplification and melt curves of patients before and after treatment. Patient A remained in long term remission after treatment, Patient B achieved remission but later recurred, and Patient C was refractory to treatment. For all patients, the Cq values at the initial visit (V1) were approximately the same (ΔCq <1.5) with or without *Lactobacillus* blocker, indicating that *Lactobacillus* was a small portion of total bacteria. However, at the post-treatment visit (V2), the remission patient has no detectable product, indicating that all or almost all bacteria were *Lactobacillus*. The refractory patient at post-treatment generated a blocked Cq of 28, a ΔCq shift

of 8 cycles indicating approximately 98% *Lactobacillus*, perhaps more importantly, 2% non-*Lactobacillus* potentially prognostic of later recurrence. The refractory patient generated a small post-treatment ΔCq approximately the same as her initial, acute BV visit, indicating >50% non-*Lactobacillus* species. Tm values at pretreatment visits vary with the patient, but tend to be above or below the 87°C range that is the signature of *Lactobacillus* species. This Tm was the only one detected in this posttreatment patient sample. It was also restored in the recurrent patient, although a non-*Lactobacillus* Tm (89°C) was detected when amplification of *Lactobacillus* was blocked. In the refractory, non-*Lactobacillus* Tm values were seen posttreatment even without blocking, indicating even higher proportions of these species than seen in the recurrent patient.

ROC and distribution analyses of the dataset in this paper resulted in optimized scoring algorithms for diagnosis of BV that maximizes sensitivity and specificity. LbRC/5 is a modified ΔCq value, in which samples with unblocked Tm values outside the 87°C range of *Lactobacillus spp.* are penalized by dividing their ΔCq value by 5.

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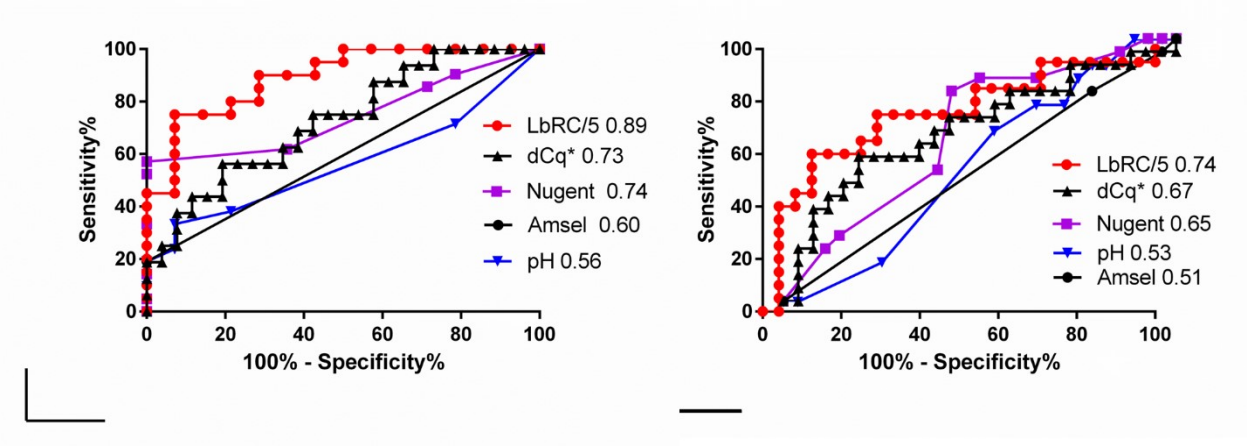

Fig. S3. ROC analysis of prognosis of treatment outcomes by four diagnostic test results at the post-treatment visit. Women were classified as recurrent patients in A, if at a later visit, they became Amsel positive and symptomatic requiring treatment, or as remission patients if they did not recur by this definition. In B, patients were classified as recurrent if at a later visit they became Amsel positive, and as remission if that did not happen. Areas under the curve are listed after each assay label.